

PRELIMINARY AMENDMENT
U.S. Application Not Yet Assigned

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0020] on page 7 with the following paragraph [0020]:

The concentration IC_{50} of nanocolloidal platinum necessary for reducing the concentration of active oxygen species such as superoxide anion radicals to half is preferably 200 mmol/L - $\mu\text{mol/L}$ or less. “Superoxide anion radicals” may be represented by O_2^- , though they are called superoxide anions in some literatures. It should be noted that the “superoxide anion radicals” used herein includes “superoxide anions.”

Please replace paragraph [0025] on page 8 with the following paragraph [0025]:

The content of the nanocolloidal platinum in the drink is preferably 0.001-100- mmol/L - $\mu\text{mol/L}$. The nanocolloidal platinum-containing drink preferably contains a cation with an osmotic pressure of 250-350 $\text{mOsm}\cdot\text{kg}^{-1}$. The cation is preferably at least one selected from the group consisting of a sodium ion, a potassium ion, a magnesium ion and a calcium ion.

Please replace paragraph [0034] on page 11 with the following paragraph [0034]:

The nanocolloidal platinum has high ability to remove active oxygen species such as superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, etc. The concentration IC_{50} of nanocolloidal platinum necessary for reducing active oxygen species to half is preferably 200 mmol/L - $\mu\text{mol/L}$ or less, more preferably 180 mmol/L - $\mu\text{mol/L}$ or less. The IC_{50} is defined herein as the minimum concentration of the nanocolloidal platinum dispersion necessary for making a ratio of C_{pt}/C_w to 50%, wherein C_w represents the concentration of active oxygen species measured 45 seconds after water is added to an equal amount of an aqueous solution generating a predetermined concentration of active oxygen species, and C_{pt} represents the concentration of

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active oxygen species measured 45 seconds after the nanocolloidal platinum dispersion is mixed with an equal amount of the same aqueous solution generating active oxygen species. It is presumed that the generated active oxygen species are mainly superoxide anion radicals. However, because other active oxygen species (hydroxyl radicals, hydrogen peroxide, etc.) can be removed, the concentration of active oxygen species means the total concentration of all active oxygen species. Because the concentration of active oxygen species cannot directly be measured, it is determined from the measured amount of a captor of an active oxygen species.

Please replace paragraph [0035] bridging pages 11 and 12 with the following paragraph [0035]:

Taking superoxide anion radicals, one of the active oxygen species, for example, the method of measuring IC₅₀ will be explained. The superoxide anion radicals can be generated by usual methods, such as an enzymatic reaction method using hypoxanthine (HXN) as a reaction substrate and xanthine oxidase (XOD) as an oxidizing enzyme, a chemical reaction method using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor and phenazine methosulfate (PMS) as an electron-transferring agent. When IC₅₀ determined in an HXN/XOD system differs from IC₅₀ determined in an NADPH/PMS system, at least one IC₅₀ is preferably 200 mmol/L μmol/L or less, and both of them are more preferably 200 mmol/L μmol/L or less.

Please replace paragraph [0037] bridging pages 12 and 13 with the following paragraph [0037]:

[2] Production method of nanocolloidal platinum dispersion

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(1) Preparation of platinum salt solution

A solution comprising a platinum-salt acid or its salt (simply called platinum salt hereafter), a polyacrylic acid salt, an alcohol and water is first prepared. The platinum salt is preferably soluble in a mixed solvent of an alcohol and water. Such platinum salts may be, for instance, hexachloroplatinic acid, potassium hexachloroplatinate, etc. Among them, hexachloroplatinic acid is preferable.

Please replace paragraph [0050] bridging pages 16 and 17 with the following paragraph [0050]:

[3] Nanocolloidal platinum-containing drinks

(1) Concentration of platinum

The concentration of nanocolloidal platinum in the nanocolloidal platinum-containing drinks is preferably 0.001-100-mmol/L μmol/L, more preferably 0.01-10-mmol/L μmol/L.

When the concentration of nanocolloidal platinum is less than 0.001-mmol/L μmol/L, one should inefficiently have a large amount of a nanocolloidal platinum-containing drink to take a sufficient amount of nanocolloidal platinum. Even if the concentration exceeds 100-mmol/L μmol/L, a correspondingly improved antioxidant effect cannot be obtained, undesirable in terms of cost.

Please replace paragraph [0063] on page 21 with the following paragraph [0063]:

How much nanocolloidal platinum-containing drink should be taken depends on the age of a person having the drink, the purpose of having the drink, the concentration of nanocolloidal platinum, etc. When a usually active adult person takes about 1-100 mL/kg/day of the drink

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having a nanocolloidal platinum concentration of about 0.1-mmol/L μmol/L, active oxygen species are extremely effectively removed from his or her body.

Please replace paragraph [0068] bridging pages 22 and 23 with the following paragraph [0068]:

- (2) Measurement of antioxidant ability
 - (a) Hypoxanthine (HXN) / xanthine oxidase (XOD)
 - (i) Measurement of amount of active oxygen species captor

Using HXN as a reaction substrate and XOD as an oxidizing enzyme, active oxygen species (simply expressed by O_2^-) were generated by an enzymatic reaction, to cause a reaction with the aqueous PAA-Pt dispersion as described below. First, 1 mmol/L of the aqueous PAA-Pt dispersion prepared in the above step (1) was diluted by Milli-Q water to 25-mmol/L μmol/L, 50 mmol/L μmol/L, 75-mmol/L μmol/L, 100-mmol/L μmol/L, 125-mmol/L μmol/L, 150 mmol/L μmol/L, 175 μmol/L and 200-mmol/L μmol/L, respectively, as shown in Fig. 1. Dissolved in 100 mL-μL of each diluted dispersion were 50 mL-μL of an aqueous HXN solution [5.5 mmol/L, containing 200 mmol/L of a phosphoric acid buffer (pH 7.5), HXN: available from Wako Pure Chemical Industries, Ltd.], and 15 mL-μL of 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, available from Dojindo Laboratories). 50 mL-μL of an XOD solution [0.2 U/mL, containing 200 mmol/L of a phosphoric acid buffer (pH 7.5), XOD: available from Roche] was added to the resultant aqueous dispersion of HXN and PAA-Pt containing DMPO, to form an aqueous HXN-PAA-Pt-XOD dispersion containing DMPO. To prevent the deactivation of XOD, the XOD-containing solution was cooled in an ice bath.

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Please replace paragraph [0074] bridging pages 24 and 25 with the following paragraph [0074]:

50 $\text{mL-}\mu\text{L}$ of aqueous solution of NADPH (available from Wako Pure Chemical Industries, Ltd.) [concentration: 13.3 mmol/L, containing 200 mmol/L of a phosphoric acid buffer (pH 7.5)] was used as an electron donor, and 50 $\text{mL-}\mu\text{L}$ of aqueous solution of PMS (available from Wako Pure Chemical Industries, Ltd.) [concentration: 17.6- $\text{mmol/L }\mu\text{mol/L}$, containing 200 mmol/L of a phosphoric acid buffer (pH 7.5)] was used as an electron-transferring agent. 100 $\text{mL-}\mu\text{L}$ of the aqueous PAA-Pt dispersion at each concentration of Pt shown in Fig. 1 was mixed with an aqueous NADPH solution, and the resultant aqueous NADPH-PAA-Pt dispersion was mixed with DMPO and finally with an aqueous PMS solution in the same manner as in the above step (a). The amount of DMPO-OOH in the resultant aqueous NADPH-PAA-Pt-DMPO-PMS dispersion was measured using ESR after 45 seconds from the addition of the aqueous PMS solution. Because PMS is decomposable by light, a series of analyses was conducted in the dark.

Please replace paragraph [0075] on page 25 with the following paragraph [0075]:

Using as a control the amount of the O_2^- captor when DMPO was added to an aqueous solution containing only the aqueous NADPH solution and Milli-Q water (Pt concentration: 0 $\text{mmol/L }\mu\text{mol/L}$), the percentage of O_2^- remaining in the aqueous solution was calculated from the percentage of the O_2^- captor to the control at each Pt concentration. The percentage of the remaining O_2^- at each concentration of Pt is shown by white circles in Fig. 1.

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Please replace paragraph [0076] on page 25 with the following paragraph [0076]:

The IC_{50} of PAA-Pt determined from Fig. 1 was $135.7 \pm 9.0 \text{ mmol/L} \mu\text{mol/L}$ in the HXN/XOD system, and $45.6 \text{ mmol/L} \mu\text{mol/L}$ in the NADPH/PMS system. The IC_{50} of PAA-Pt in the HXN/XOD system is shown in Table 2.

Please replace paragraph [0084] on page 29 with the following paragraph [0084]:

Comparative Example 2

The IC_{50} of vitamin C was determined in the same manner as in the (2)(a) in Example 1, except for adding an aqueous solution of vitamin C (L-ascorbic acid, available from Wako Pure Chemical Industries, Ltd., $100 \text{ mmol/L} \mu\text{mol/L}$) to an aqueous HXN/XOD solution generating O_2^- . The results are shown in Table 2.

Please replace paragraph [0085] on page 29 with the following paragraph [0085]:

An aqueous solution of NADPH, vitamin C and PMS was prepared in the same manner as in the step (2)(d) in Example 1, except for adding NADPH and PMS to an aqueous solution of vitamin C (L-ascorbic acid, $100 \text{ mmol/L} \mu\text{mol/L}$), and the amount of O_2^- remaining in the solution was determined. The change with time of the percentage of the remaining O_2^- from the addition of PMS in the preparation of the aqueous solution of NADPH, vitamin C and PMS is shown by black circles in Fig. 2.

Please replace paragraph [0089] bridging pages 30 and 31 with the following paragraph [0089]:

Reference Example 1

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A nanocolloidal platinum dispersion with polyvinylpyrrolidone (PVP) as a protecting agent (aqueous PVP-Pt dispersion, R value: 40) was prepared in the same manner as in the step (1) in Example 1 except for using 0.1467 g of PVP as a protecting agent. The IC₅₀ of PVP-Pt was then measured in the same manner as in the steps (2)(a) and (c) in Example 1 except for adding the aqueous PVP-Pt dispersion to aqueous HXN/XOD solution generating O₂[·]. As a result, the IC₅₀ was 148.7 ± 23.2 ~~mmol/L~~ μmol/L.

Please replace paragraph [0093] bridging pages 31 and 32 with the following paragraph [0093]:

Comparative Example 4

Measurement revealed that “Hakkin-Gensui” (tradename of The Lead Cooperation And Association) commercially available as a platinum-containing water had a Pt concentration of 2.5 mmol/L. The IC₅₀ of “Hakkin-Gensui” was measured in the same manner as in the step (2)(a) in Example 1, except for adding “Hakkin-Gensui” at various concentrations to an aqueous HXN/XOD solution generating O₂[·]. As a result, the IC₅₀ was 251.4 ± 7.4 ~~mmol/L~~ μmol/L. Also, the oxidation-reduction potential of “Hakkin-Gensui” was 470 ± 2.3 mV. The results are shown in Table 3.

Please replace paragraph [0096] on page 33 with the following paragraph [0096]:

However, Reference Example 1 indicates that all having low oxidation-reduction potential do not necessarily have large ability to remove active oxygen species. Namely, the PVP-Pt of Reference Example 1 has as small IC₅₀ as 148.7 ± 23.2 ~~mmol/L~~ μmol/L despite as high oxidation-reduction potential as 629 ± 0.9 mV, while “Hakkin-Gensui” of Comparative

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Example 4 has as large IC₅₀ as 251.4 ± 7.4 ~~mmol/L~~μmol/L despite its oxidation-reduction potential of 470 ± 2.3 mV lower than that of the PVP-Pt of Reference Example 1.